Construction and characterization of chromosome 1B specific DNA library of wheat

Fa-Yun Zhang[1,2,6], Wei-Bo Yin[1,5], Rui Shi[1], Ying-Kao Hu[2], Yue-Ming Yan[2,4], Yu-Hong Chen[1], Yi-Hua Zhou[1], Jun Hu[1], Richard R.-C. Wang[3], and Zan-Min Hu[1,4]

[1]*Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences, Beijing 100101, China;* [2]*Biological Department, Capital Normal University, Beijing 100037, China;* [3]*USDA-ARS, FRRL, Utah State University, Logan, UT84322-6300, USA.* *Received 22 January 2004, accepted 11 October 2004.*

Zhang, F-Y., Yin, W-B., Shi, R., Hu, Y-K., Yan, Y-M., Chen, Y-H., Zhou, Y-H., Hu, J., Wang, R. R.-C. and Hu, Z-M. 2005. **Construction and characterization of chromosome 1B specific DNA library of wheat**. Can. J. Plant Sci. **85**: 309–316. Chromosome 1B was microdissected and collected from chromosome spreads of wheat (*Triticum aestivum* L. 'Jing 411'), using a glass needle. DNA of the isolated chromosome was amplified in vitro by *Sau*3A linker adaptor-mediated polymerase chain reaction (LA-PCR). The second-round PCR products were verified by Southern hybridization using DIG-labeled genomic DNA of wheat. The results initially showed the DNA was from wheat genome. A pair of SSR primers specific to chromosome 1B was used to verify the origin of the PCR products from the isolated chromosome. The results confirmed that the PCR products originated from chromosome 1B. The second round of PCR products from chromosome 1B were cloned into plasmid pUCm-T vectors to create a chromosome-specific library, which included approximately 248 000 recombinant clones. Characterization of 100 randomly selected clones of the library showed that the insert size ranged between 0.5 and 2.0 kb, with an average of 1 kb. Randomly selected 288 clones were characterized with dot blot hybridization, of which 57.2% were medium/high copy clones and 42.8% low/single copy clones. The application of this technique to establish high-density molecular maps for chromosome 1B is discussed.

Key words: Wheat, chromosome microdissection, chromosome-specific library

Zhang, F-Y., Yin, W-B., Shi, R., Hu, Y-K., Yan, Y-M., Chen, Y-H., Zhou, Y-H., Hu, J., Wang, R. R-C. et Hu, Z-M. 2005. **Constitution et caractérisation d'une banque génétique spécifique au chromosome 1B du blé**. Can. J. Plant Sci. **85**: 309–316. Les auteurs ont recueilli le chromosome 1B de plants de blé (*Triticum aestivum* L. 'Jing 411') avec une aiguille de verre avant de le disséquer. L'ADN du chromosome isolé a ensuite été amplifié *in vitro* par la réaction en chaîne de la polymérase au moyen du lieur *Sau3*A (LA-PCR). Les produits obtenus au deuxième tour de PCR ont été vérifiés par la méthode d'hybridation de Southern après marquage de l'ADN du blé à la digoxygénine. Les premiers résultats indiquaient que l'ADN provenait bien du génome du blé. On s'est servi d'une paire d'amorces SSR spécifiques au chromosome 1b pour vérifier l'origine des produits de la PCR issus du chromosome isolé. Les résultats confirment que les produits de la PCR viennent du chromosome 1B. Les produits du deuxième tour de PCR venant du chromosome 1B ont été clonés avec les vecteurs du plasmide pUCm-T pour donner une banque spécifique à ce chromosome comprenant 248 000 clones issus de la recombinaison. La caractérisation de 100 clones sélectionnés au hasard dans cette banque révèle que la taille du fragment inséré varie de 0,5 à 2,0 kb, pour une moyenne de 1 kb. On a sélectionné au hasard 288 clones qu'on a ensuite caractérisé par hybridation sur tache. Sur ce nombre 57,2 % étaient des clones de copie moyenne/élevée et 42,8 % des clones de copie faible/simple. Suit une discussion sur l'application de cette technique à la création de cartes moléculaires pour le chromosome 1B.

Mots clés: Blé, microdissection de chromosomes, banque spécifique à un chromosome

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. Molecular markers are an efficient tool to study the genetics, the genome constitutions and wheat improvement (RÖder et al. 1998). Restriction fragment length polymorphisms (RFLP), one of the earliest applied markers, have been used to build genetic linkage maps of wheat (Liu et al. 1990; Devos and Gale 1993; Somers et al. 1996). Hexaploid bread wheat has an extremely large genome of 16×10^9 bp/1C of which more than 80% is repetitive DNA (Stephenson et al. 1998). It is labor-intensive to obtain new molecular markers and to saturate the linkage map of a known specific chromosome (or region) by using the conventional procedures of sampling from cDNA

[4]*To whom correspondence should be addressed (e-mail: zmhu@genetics.ac.cn).*
[5]*Author who had the same contribution to the research as the first author.*
[6]*Laboratory of Photosynthesis Basic Research, Institute of Botany, Chinese Academy of Sciences.*

Abbreviations: **LA-PCR**, *Sau*3A linker adaptor-mediated polymerase chain reaction; **RFLP**, restriction fragment length polymorphisms

or genomic DNA libraries (Moore et al. 1993). A direct approach to resolve this problem is to establish chromosome-specific DNA libraries using chromosome microdissection and microcloning. A number of useful probes selected from these libraries can be used to construct the genetic maps and high-density physical maps. Since 1981, the microdissection and microcloning techniques have made notable progress in the diagnosis of chromosomal disease in humans (Tigaud et al. 1996); construction of chromosomal specific DNA libraries of plants, such as *Hordeum vulgare* (Schondelmaier et al. 1993), *Avena` sativa* (Chen and Armstrong 1995), soybean (Zhou et al. 2001); gene localization and cloning, for example *Sry* gene from the Y chromosome of *Muntiacus muntjak* (Zhang et al. 2001); and studies on disease molecular genetics, such as human malignant melanoma (Guan et al. 2002) by combining with molecular and cellular techniques.

There are many important genes on chromosome 1B of wheat, such as high molecular weight glutenin subunit (HMW-GS), low molecular weight glutenin subunit (LMW-GS), and gliadin genes (Payne 1987), stripe-rust resistance genes *Yr*9, *Yr*H52 and *Yr*15 (Peng et al. 2000; Shi et al. 2001) and resistance to fusarium head blight (FHB) (Buerstmayr et al. 2002). To date, there has been no report of a chromosome 1B specific DNA library. We report the construction and characterization of a chromosome 1B specific DNA library by using chromosome microdissection and microcloning techniques.

MATERIALS AND METHODS

Materials

The seed of the cultivar Jing 411 of wheat (*Triticum aestivum* L.), kindly provided by Dr. Yueming Yan, Capital Normal University, Beijing, was used in the experiments.

Methods

Chromosome Preparation

The wheat seeds were immersed in warm water (25°C) for 5–8 h, then germinated on moist filter paper in a Petri dish at 25°C in the dark. After the seeds sprouted, they were cultured at 4°C for 24 h, then at 25°C in the dark until the roots grew up to 0.5–1 cm. The seeds with roots (0.5–1 cm) were treated in ice water (0°C) for 24 h to increase metaphase cells. Then root tips were harvested and fixed in 3:1 ethanol: acetic acid for 5 min, and transferred immediately into 70% (vol/vol) ethanol and stored at –20°C. Before being squashed, the root tips were digested with an enzyme mixture of 2.5% (wt/vol) cellulase (Onozuka RS; Yakult Pharmaceutical, Japan) and 2.5% (wt/vol) pectolase (Y-23; Kyowa Chemical, Japan) in 75 mM KCl, 7.5 mM EDTA at 37°C for 15–25 min, then rinsed in ddH_2O and stored at 4°C for 15–20 min. After the root tips were squashed in a drop of 1% (wt/vol) Carbol Fuchsin solution, they were immediately used for microdissection.

Chromosome Microdissection

Chromosome microdissection was done by using a manually made glass needle with a tip of 1–3 μm fixed on the arm of a LeitZ micro-operation instrument on an inverted microscope. The chromosome samples with well-dispersed mitotic metaphase were used and the target chromosome (i.e., chromosome 1B) was microdissected and collected by using the fixed glass needle according to the method described by Hu et al. (1998). The glass needle tip with dissected chromosome 1B was broken into an Eppendorf tube containing 20 μL of proteinase K solution (19 ng μL^{-1}; Roche, Germany) in 1 × T4 ligase buffer, 30 mM Tris-HCl, pH 7.8 at 25°C, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP (Promega, USA). Subsequently, the Eppendorf tubes were centrifuged (10 600 × *g*, 10 s) and stored at –20°C.

Preparation of Sau3A Linker Adaptors and the DNA Amplification of the Microdissected Chromosome

After microdissection, the isolated chromosome DNA were amplified using Sau3A Linker adaptor mediated PCR (LA-PCR) following protocols described by Chen and Armstrong (1995), and Zhou et al. (1999). In brief, the 23 mer DNA sequence 5'-GATCCTGAGCTCGAATTCGAC-CC-3' and the 19 mer DNA sequence 5'-GGGTC-GAATTCGAATTCGAGCTCAG-3' were synthesized (Cybersyn Company, China) and *Sau*3A linker adaptors were prepared as described by Chen and Armstrong (1995). After the isolated chromosome was treated at 37°C for 2 h in a Proteinase K solution (19 ng μL^{-1}; Roche, Germany), the chromosomal DNA was digested with 0.02U *Sau*3A (Promega, USA) at 37°C for 2h. The digested chromosomal DNA was linked with *Sau*3A adaptor (2 μL, 5 ng μL^{-1}) using T4 DNA 1igase (0.5 μL, 3 U μL^{-1}; Promega, USA) in a total volume of 24.5 μL at 16°C overnight. All the enzymes used in above mentioned procedures were inactivated at 70°C for 20 min after the reactions. Two rounds of PCR were performed. The first round of PCR was carried out in the same tube by adding 10 μL of 10 × Taq buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0 at 25°C, 1% Triton X-100 (wt/vol) and 15 mM $MgCl_2$. Promega, USA), 6 μL of 25 mM $MgCl_2$, 2 μL of 10 mM dNTPs, 1 μL of 19 mer primer (50 ng μL^{-1}), 2U Taq DNA polymerase (Promega, USA) and double distilled water in 100 μL total volume. After denaturing at 94°C for 5 min, amplification was performed with 35 cycles of 1 min at 94°C, 1.5 min at 50°C, 3 min at 72°C, followed by a final 15 min extension at 72°C. The second round of PCR was done under the same conditions described above except that only a 2 μL product from the first round of PCR was used as the template. In all the procedures, strict positive and negative control experiments were carried out using the same conditions but the templates. In a positive control experiment, 10 pg of wheat genomic DNA was used as the template, whereas no DNA template was used in the negative control.

Southern Hybridization Analysis

Wheat genomic DNA was extracted from young leaf tissue according to the protocol developed by Fu et al. (1994). Briefly, young leaves (1 g) of wheat were collected from wheat plants (three-leaf stage) in a greenhouse, frozen in liquid nitrogen and quick ground into granules. The leaf granules were mixed with 4 mL extraction solution of 2 × CTAB (2% CTAB, wt/vol, 20 mM EDTA, 100 mM Tris-HCl, pH

8.0, 1.4 M NaCl) and the mixture was vortexed slowly and incubated at 65°C for 1 h. The DNA was extracted twice with phenol and once with chloroform/isopropanol (24:1, vol/vol). A two-third volume of isopropanol was used to precipitate the DNA. Finally the genomic DNA precipitate was resuspended in 0.5 mL of TE.

The second round PCR products from chromosome 1B and 10 μg *Eco*R digested wheat genomic DNA were separated on 1.5% agarose gel (agrose/TAE buffer, pH 8.5) and transferred onto the nylon membrane (Hybond$^+$; Amersham, USA). Probe labeling, Southern hybridization and detection were carried out according to the manufacturer's instruction of DIG DNA labeling and detection Kit (Roche, Germany). In brief, for DIG DNA labeling, wheat genomic DNA (2.5 μg) digested with *Eco*RI was diluted to a total volume of 15 μL, denatured for 10 min and quickly chilled on ice, then mixed with 2 μL hexanucleotide mix, 2 μL dNTP mixture (1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM DIG-dUTP) and 2U Klenow enzyme. The mixture was centrifuged briefly at 10 600 × *g* and incubated overnight at 37°C. After 2 μL of 0.2 M EDTA (pH 8.0) was used to stop the reaction, the labeled DNA was precipitated by adding 2.5 μL of 4 M LiCl and 75 μL prechilled (–20°C) ethanol and the DNA pellet was dissolved in 30 μL distilled water. For Southern blot hybridization, standard hybridization buffer (20 mL 100 cm^{-2}, 5× SSC, 0.1% N-lauroylsarcosine, 0.02% SDS, 1% blocking solution) and denatured DIG-labeled DNA probe (30 ng 100 cm^{-2}) were incubated at 65°C for 16 h. The hybridized DNA fragments on nylon membrane were detected with DIG-DNA Detection System (Roche, Germany) using anti-DIG-AP conjugate (150 mU mL^{-1}, 20 mL 100 cm^{-2}) and color-substrate solution (10 mL 100 cm^{-2}).

Verification of the Origin of the Amplification Products

Two microsatellite primers, 5' GGTGGTATGGACTATGGACACT 3' (22mer) and 5' TTTGCATGGAGGCACATACT 3' (20mer) for the *Xgwm498* microsatellite, which is located on chromosome 1B of wheat (RÖder et al. 1998), were used to identify the origin of PCR products. Approximately 80 ng of wheat genomic DNA or 2 μL of the second-round PCR products from chromosomal samples were used as the template. PCR amplification was performed by using 2 μL of 10 × Taq buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0 at 25°C, 1% Triton X-100 and 15 mm $MgCl_2$), 0.2 μL of 10 mM dNTP, 2 μL of 2 μM primers, 0.8U of Taq DNA polymerase (Promega, USA), and distilled water in a total volume of 20 μL. After denaturation at 94°C for 1 min, the amplification was done with 35 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, followed by a final extension at 72°C for 5 min. *Xpsp3009* microsatellite that is located on chromosome 6B of wheat (Stephenson et al. 1998) was detected by PCR using the second round PCR products from chromosome 1B as the template with the procedure described by Stephenson et al. (1998).

Library Construction and Analysis of Plasmid Clones

The products of the second round PCR were purified with the UltraPure™ PCR Product Purification Kit (SBS GenoTech Co., Ltd., China) according to the manufacturer's instruction. 100 μL of the second round PCR products were added to an Eppendorf tube containing 400 μL of resinous solution. After complete mixing, the mixture was transferred to a column and centrifuged at 17 900 × *g* for 30 s. The column was washed twice with 80% ethanol by centrifugation at 17 900 × *g* for 2 min. 10 μL of TE buffer for dissolving DNA was added to the column. The purified PCR products were collected in a new Eppendorf tube by centrifugation at 17 900 × *g* for 30 s. 2 μL of purified DNA (100 ng) was ligated into pUCm-T vectors (Sangon BioTech, China) in 10 μL volume at 4°C for 16 h. One-tenth volume (1 μL) of ligation mixture was used for transformation of DH5 α competent *E. coli* cells (200 μL) by heat shock (42°C for 90 s). After 1 h of growth in 800 μL of LB medium at 37°C, 100 μL of transformed DH5 α cells were spread on the LB plate containing ampicillin, X-gal and isopropyl-1-thio-galactopyranoside (IPTG). Plasmid DNA from 100 randomly selected recombinants (white clones) from the library was extracted by alkaline lysis. The inserts were released by digesting with *Pst*I and separated in a 1.2% agarose gel (agarose/TAE buffer, pH 8.5, wt/vol). 288 recombinant plasmid DNA (each 120 ng) were applied onto Hybond$^+$ filters and hybridized with DIG-labeled wheat genomic DNA to estimate the copy number of the inserts. According to the intensity of dot-blot hybridization signals, inserts were estimated to be low/unique copy (weak or no signals) and medium/high copy (strong signals) sequences (Zhou et al. 2001). The dot blot hybridization was performed according to the manufacturer's instruction of DIG DNA labeling and detection Kit (Roche, Germany).

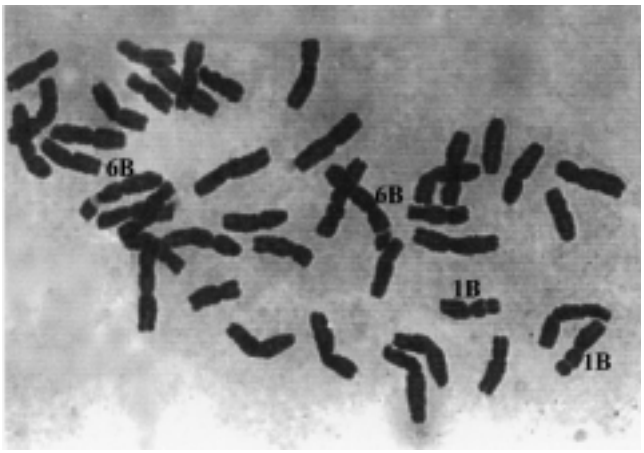
RESULTS AND DISCUSSION

Microdissection of Chromosome 1B

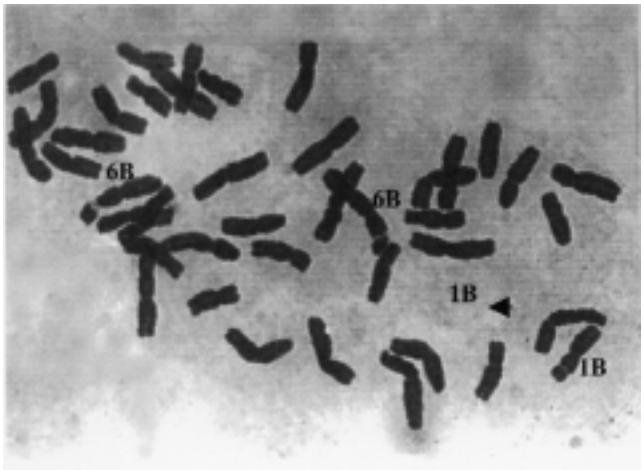
Chromosome 1B and 6B of wheat can be easily distinguished from other chromosomes based on the presence of satellites. Chromosome 1B differs from chromosome 6B in size. A single chromosome 1B of wheat (Fig. 1A), which was identified by its smaller size than that of 6B and its satellite, was microdissected and collected by using a microglass-needle (Fig. 1B, 1C), then transferred into a 0.2 mL microcentrifuge tube containing proteinase K.

Amplification of Chromosome 1B and Southern Blot Analysis

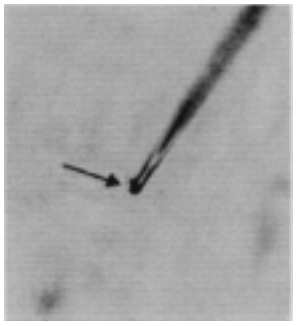
After digestion with proteinase K and restriction enzyme *Sau*3A, the chromosome 1B DNA was ligated to *Sau*3A linker adaptors, then amplified with two rounds of LA-PCR. The size of the second round PCR products ranged from 0.3 to 2.5 kb (Fig. 2A, lanes 3–5). The positive control, using 10 pg of genomic wheat DNA as template, had a brighter and wider band ranging from 0.3 to 3.5 kb in size (Fig. 2A, lane 2). No product was amplified from the negative control (Fig. 2A, lane 6), which contained no template DNA but was used for monitoring possible contamination. The wheat genomic DNA probe labeled with DIG hybridized with the amplified products, resulting in signals from those lanes containing digested genomic DNA, positive control, and amplified products from chromosome 1B (Fig. 2B, lanes 2–5) and



1A



1B



1C

Fig. 1. Chromosome spreads of wheat mitotic metaphase chromosomes from root tip cell used for chromosome isolation. A. Mitotic metaphase chromosomes (1025 ×) of a root tip cell of wheat before chromosome isolation. B. Mitotic metaphase chromosomes (1025 ×) after isolation of the chromosome 1B (the arrow indicates the position of the isolated chromosome 1B). C. The isolated chromosome 1B (500 ×) was adhered to the tip of a glass needle.

indicating that the microdissected chromosomes came from the wheat genome.

Although the microdissection and microcloning techniques had been established since 1981, their application and development have followed the advance of the PCR technology. Ludecke et al. (1989) initially established a method of vector-mediated PCR amplification of microdissected chromosome. To date, several methods of PCR amplification of microdissected chromosome have been designed, among which the LA-PCR and degenerated oligonucleotide primed-PCR (DOP-PCR) were the most widely used (Albani et al. 1993; Chen and Armstrong 1995; Dang et al. 1998; Zhou et al. 2000). With LA-PCR, amplification of contaminated foreign DNA could be more effectively avoided than that with DOP-PCR, because contaminated DNA could not be amplified unless the DNA contamination occurred before adaptors were linked with the microdissected chromosome DNA (Dang et al. 1998; Zhou et al. 1998). To avoid possible DNA amplification of contaminants, a relatively high annealing temperature (50°C) was used in our research. More importantly, we isolated a single chromosome 1B of wheat and amplified its DNA using LA-PCR method, which significantly reduced the contamination

Verification of the Origin of PCR Products Using Microsatellite Markers

The microsatellite marker *Xgwm498*, which is located on wheat chromosome 1B, was used for PCR amplification. A 167 bp band resulted from PCR reactions when wheat genomic DNA (Fig. 3, lane 1), amplified DNA from chromosome 1B (Fig. 3, lane 3) and PCR products of positive control (Fig. 3, lane 2) were used as the template. No band was obtained in the negative control (Fig. 3, lane 0). When microsatellite marker *Xpsp3009,* located on chromosome 6B, was used for PCR amplification, no amplified product was produced from the PCR product of chromosome 1B (Fig. 4, lane 3). However, a 221 bp DNA fragment resulted from PCR reactions in which either wheat genomic DNA or PCR products of positive control were used as the template (Fig. 4, lanes 1 and 2). These results demonstrated that the amplified DNA from the microdissected chromosome was indeed from chromosome 1B of wheat. The results also showed that the amplified DNA from the microdissected chromosome contained abundant simple sequence repeats and had potential use for exploring chromosome 1B specific microsatellite markers.

The key step in microdissection and microcloning is to verify the origin of the microdissected chromosome. In previous reports, genomic DNA was labeled as the probe and hybridized with the PCR products from the isolated chromosome to confirm the chromosome's origin (Jung et al. 1992; Albani et al. 1993, Chen and Armstrong 1995). In our research, besides using Southern blotting, we employed a chromosome 1B specific microsatellite marker (*Xgwm498*) to identify the amplified products of microdissected chromosome. Cheng et al. (1998) successfully confirmed the amplification products from the short arm of rice chromosome 5 using the mapped SSRs of rice. Therefore, mapped SSRs could be used as a useful tool to verify the origin of PCR products of microdissected chromosomes.

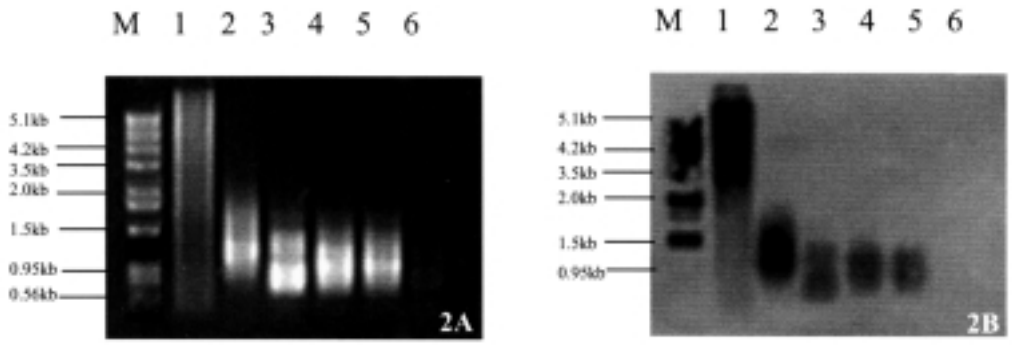


Fig. 2. Products of the second round of PCR using microdissected chromosome 1B DNA of wheat as templates and linker adaptors as primers (2A, viewed in an Ethidium bromide stained gel), and the characterization of the product by Southern hybridization using wheat genomic DNA labeled with DIG as the probe (2B, viewed as a chromatograph). Lane 1: The wheat genomic DNA digested by *Eco*RI; Lane 2: Positive control (LA-PCR products using wheat genomic DNA as the template); Lane 3–5: The second round PCR products of chromosome 1B; Lane 6: Negative control without any template DNA; M: λ DNA digested with *Hind*III/ *Eco*RI.

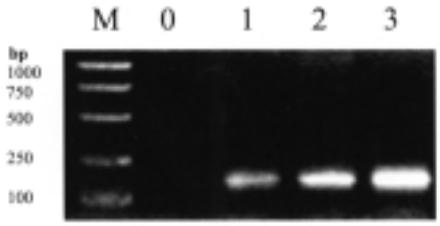


Fig. 3. Ethidium bromide stained gel image for verification of DNA amplified from microdissected chromosome 1B using microssatellite markers located on chromosome 1B (*Xgwm498*). Lane 0: Negative control; Lane 1: Wheat genomic DNA as the template; Lane 2: Positive control (using PCR product from positive control of LA-PCR as the template); Lane 3: The second round PCR product from chromosome 1B DNA as the template; Lane M. DGL2000 DNA size marker (Beijing Dingguo BioTech Co., Ltd., China).

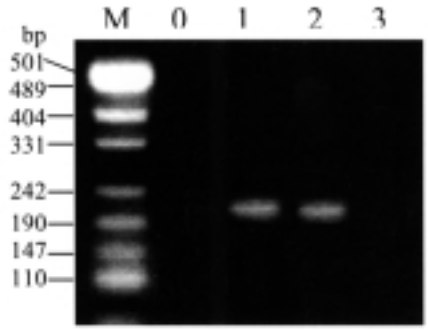


Fig. 4. Ethidium bromide stained gel for verification of DNA amplified from microdissected chromosome 1B using microssatellite markers located on chromosome 6B (*Xpsp3009*). Lane 0: Negative control; Lane 1: Wheat genomic DNA as the template; Lane 2: Positive control (using PCR product from positive control of LA-PCR as the template); Lane 3: The second round PCR product from chromosome 1B DNA as the template; Lane M: pUC19 DNA digested with *Msp*I (*Hpa*II).

Recently, the potentiality of selecting microsatellite markers following chromosome microdissection and microcloning has attracted more attention (Busch et al. 1995; Zhang et al. 1998; Zheng et al. 1998). Our research showed that SSRs could be obtained from PCR product of specific chromosomes. Bryan et al. (1997) pointed out that to acquire microsatellite markers, the genomic DNA should be digested by an enzyme sensitive to methylated bases, such as *Sau*3A. The reason was that the enzyme sensitive to methylation could exclude a majority of highly repeated sequences and enrich SSRs. It is clear that *Sau*3A linker adaptor-mediated PCR amplification of microdissected chromosome DNA is suitable to obtain microsatellite sequences.

Construction and Characterization of the Specific DNA Library from Chromosome 1B

The second-round LA-PCR products from chromosome 1B were cloned into the pUCmT-vector, which had two T-extrusions on the 3' ends of the inserted site. This special structure not only improved the ligation rate extremely well but also prevented self-ligation effectively. A total of 496 recombinant clones were obtained from a small fraction (1/500) of transformed DH5 α cells of the second round PCR products. It was estimated that approximately 2.48×10^5 recombinant clones could be present in the DNA libraries, if the clone redundancy was not considered. Examination of 100 white clones randomly selected from the library showed that the insert sizes varied from 500 to 2000 bp with a calculated average of 1000 bp (Fig. 5). Dot blot hybridization of 288 recombinant clones to DIG-labeled genomic DNA of wheat showed that 42.8% of the clones had weak or no hybridization signals, which represented low/unique copy sequences, and 57.2% of the clones gave strong hybridization signals, which were of medium or highly repetitive sequences (Fig. 6).

One of the objectives of microdissection and microcloning of a specific chromosome is to construct a chromosome-specific library. Generally, it is expected that larger inserts could be obtained from the constructed library. Such a chromosome library is favorable for selection of RFLP probes and isolation of important genes. We established a

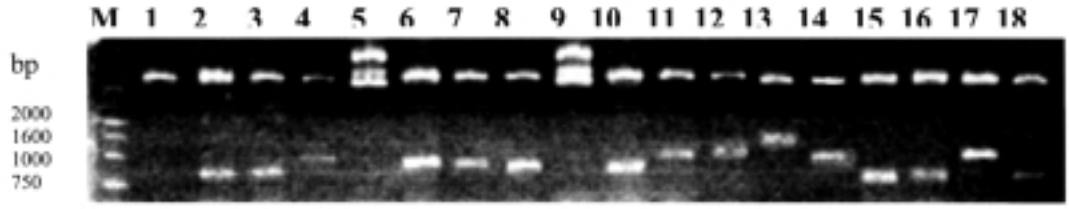


Fig. 5. Characterization of a part of recombinant clones from the DNA library of chromosome 1B. 1–18: Plasmid DNA of recombinant clones restricted by *Pst*. 5 μg of DNA was loaded in each lane. M: DGL2000 DNA size marker (Beijing Dingguo BioTech Co., Ltd., China).

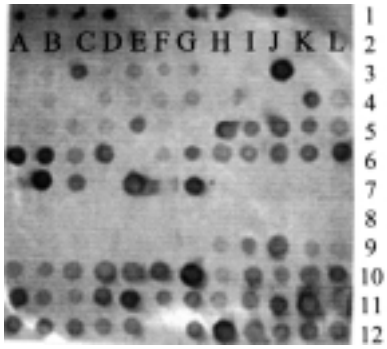


Fig. 6. Characterization of some of recombinant clones from the DNA library of chromosome 1B by dot blot hybridization using DIG-labeled wheat genomic DNA as probes. Each dot contains 120 ng of recombinant plasmid DNA.

DNA library of wheat chromosome 1B with an insert size ranging from 500 to 2000 bp, with an average of 1000 bp. While Schondelmaier et al. (1993) constructed a library for the short arm of chromosome 1H of barley, of which inserts ranged from 70 to 1150 bp with an average of 250 bp. Chen and Armstrong (1995) established a single chromosome library of oat using *Sau*3A adaptor-mediated cloning method. The size of the inserts varied from 150 to 1700 bp with an average of 650 bp. Compared to the above-mentioned reports, the inserts reported in this paper were longer. The possible explanation is that long exposure to acids, such as acetic acid, can cause depurination of chromosomal DNA. In this research we fixed the root tips only for 5 min. In addition, the insert size of clones might be related to the structure of chromosome DNA. The quality of the chromosome library is also influenced by the enrichment of low/unique copy. Wheat has an extremely large genome of which more than 80% is repetitive DNA (Stephenson et al. 1998). Low/unique copy sequences that spread over unmethylated regions in the genome might be preferentially selected by the use of the restriction enzyme *Sau*3A, which is cytosine methylation sensitive (Cheung et al. 1992). In the chromosome 1B library, the medium or highly repetitive sequences was 57%, while the low/unique copy sequences was 42%, suggesting that it had selected against repetitive sequences.

Wheat is a polyploid plant with a rather large genome. It is extremely difficult to select low/unique copy probes from cDNA or genomic DNA library for RFLP analysis. In this study, we established the chromosome 1B DNA library, from which several low/unique sequences could be obtained and applied to select the probes for RFLP analysis. In previous reports, the proportion of polymorphic markers selected from microdissected chromosomal library was 2–6% (Schondelmaier et al. 1993; Liu et al. 1997; Stein et al. 1998). Selecting molecular markers from the cDNA or gDNA library was extremely time consuming and expensive, while obtaining single copy probes (SCPs) from the chromosome-region-specific DNA library was a direct and quick way to achieve the goal (Stein et al. 1998; Liu et al. 1999). The constructed chromosome 1B DNA library can be used to select chromosome 1B specific probes. The potential application of chromosome 1B specific library is likely for new marker discovery, molecular mapping to increase the density of maps, and finally cloning the agronomically important genes located on chromosome 1B. In the chromosome 1B specific library, a number of DNA fragments homoeologous with those on chromosome 1A and 1D of hexaploid wheat will also be present. These could complicate the development of high-density map for chromosome 1B.

Chromosome-specific DNA libraries could also be prepared from flow-sorted chromosomes in wheat (Wang et al. 1992). Improved methods for chromosome and chromosome arm sorting have been developed (Vrana et al. 2000, Kubalakova et al. 2002), and may be used to obtain large quantities of chromosome-specific DNA libraries. DNA of sorted chromosome was reported to be high molecular weight (Simkova et al. 2003) and thus suitable for construction of chromosome- and chromosome-arm-specific large insert DNA libraries cloned in a BAC vector. However, the flow cytometry methods cannot be used to construct fine chromosome region-specific DNA libraries. Up to now, most chromosomes of plants cannot be sorted by flow cytometry. The approach we used here should facilitate the analysis of single chromosomes of plants.

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